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Abstracts

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Natural killer activity in kidney allograft recipients and in uremic hemodialyzed patients: In vitro effect of alfa-interferon. *C. Ausiello, M. Valeri, A. Piazza, D. Adorno, B. Evangelisita, and C.U. Casciani, Instituto di V^a Patologia Chirurgica, Università, Rome, Italy.* Several data indicate that natural killer (NK) cells may play a role, in vivo, against the outgrowth of malignant cells. Interferon (IFN) is the most conspicuous regulatory agent capable of increasing NK activity. The aim of this work was to analyze the spontaneous and IFN-induced NK activity both in corticosteroid-treated kidney allograft recipients where a significantly higher risk of malignancies is present and in uremic hemodialyzed patients which show an aspecific immunodepression. NK activity on 51-Cr labeled K562 target cells was studied in 19 functioning allograft recipients. The time that elapsed between the graft and the test varied from 4 days to 13 years. Several patients were tested more than once. Eighteen hemodialyzed and 18 normal individuals were also studied. Spontaneous NK cytotoxicity was decreased sharply after the graft. Depression or lack of NK activity was also observed in short-term transplantation (< 1 month), while long-term transplantations (> 2 years) showed a tendency toward normal values. The IFN relative enhancement of NK activity was the same as in control group, but the total activity was still lower, suggesting that the few NK cells are normally responsive at the IFN regulatory effect. The spontaneous and IFN-induced NK activity of hemodialyzed patients was not significantly different from the healthy control group, suggesting that renal function is not involved with NK activity.

Charcoal hemoperfusion in chronic uremia: 5 years of clinical application. *V. Bonomini, S. Stefoni, G. Feliciangeli, M. P. Scolari, A. Buscaroli, G. Mosconi, R. Prandini, and L. Colì. Istituto di Nefrologia e Dialisi, Università, Bologna, Italy.* The capacity of charcoal to absorb endogenous and exogenous toxins from the blood is well known. The major drawbacks to its wide use in chronic uremia have been poor biocompatibility and incapacity of absorbing urea, electrolytes, and water. The two problems have been solved by *(1)* coating the charcoal granules with highly biocompatible membranes and *(2)* using charcoal hemoperfusion in conjunction with hemodialysis (HD/HP programs). The first step in our experience was to evaluate biocompatibility, tolerance, and efficiency of a new hemoperfusion device. Subsequently, in a 5-year period we treated 33 patients according to the HD/HP program (about 2,000 procedures). Two main groups of patients have been included in the study: group A, 19 patients with persisting signs of uremia despite adequate and even intensive dialysis treatment (mainly recurring pericarditis, severe peripheral neuropathy, and pruritus) — combined HD/HP treatment led to improvement of symptoms in 70% of the patients, subjective and objective; group B, 14 patients, (residual 2.1 ± 0.9 ml/mm; mean diuresis 280 ml) in good clinical and metabolic condition. In these patients the combining of the sorption capacity of dialysis and hemoperfusion allowed a reduction of the dialysis time, up to the elimination of one entire procedure, for 3 to 26 months (mean, 10.7 ± 6.7). Patients' clinicometabolic conditions serially checked remained stable despite the one-third reduction in time of treatment, while personal and social rehabilitation markedly improved.

Hyperparathyroidism as cofactor of uremic myopathy: An alternative hypothesis. *D. Brancaccio, F. Cornelio, F. Dworzak, L. Morandi, G. Como, C. Galmozzi, M. Gioventù, and I. Poletti. Servizio di Nefrologia, Ospedali S. Paolo, Milano e Maggiore, Bergamo; Istituto Neurologico C. Besta, Milano; Divisione di Cardiologia, Ospedale S. Paolo, Milano, Italy.* Uremic patients undergoing regular dialytic treatment (RDT) often present clinico-biological signs of secondary hyperparathyroidism; however, spontaneous high levels of blood calcium and phosphate may be observed. This clinical status is called autonomous hyperparathyroidism (iPTH-3) and actively involves bone structure and several organs and apparatus as well. To clarify a possible relationship between iPTH-3 and myopathy we performed muscle biopsy in three patients affected by iPTH-3 presenting evidence for a progressive distal myopathy. One patient was also affected by a dilatative cardiomyopathy. Muscle specimens (500 mg) were frozen immediately and the usual battery of 14 histochemical and histoenzymatic reactions were performed on 10-μ serial sections. In addition, two selective staining (alizarine red and glyoxal bis) were also performed to detect a possible calcium deposition. The results in all patients evidenced a myopathy on a neurological basis and, in addition, diffuse microgranular calcium deposits in the arteriolar walls whose thickness was abnormally increased; a wall contraction and a reduced vascular lumen were also present. Muscle biopsy, performed 6 months after parathyroidectomy, demonstrated in one patient the total disappearance of vascular deposits and a thinner aspect of vascular walls. A dramatic improvement of his dilatative myocardiopathy was also observed. Our data seem to suggest the following conclusions: *(1)* It is presumable the uremic myopathy may also be due to ischemia secondary to reduced vascular lumen; *(2)* the small and diffuse deposits in the muscolaris mucosa may play a role in inducing the wall contraction; *(3)* as PTH can alter the calcium intra/calcium extracellular ratio, it is to be considered a real uremic toxin in terms of muscle tissue damage; *(4)* in patients on RDT a parathyroidectomy may also be indicated for a better control of a complex syndrome in which not necessarily bone tissue is mainly involved.

T cell subsets monitoring in renal transplantation: A statistical approach. *B. Brando, G. Civati, M. L. Broggi, C. Grillo, A. Perego, C. Guastoni, G. Busnach, A. E. Minetti, and L. Minetti. Divisione di Nefrologia, Ospedale Niguarda-Ca' Granda, Milano, Italy.* We serially monitored 64 renal transplant recipients to study the patterns of T cell subset numbers and functions in different clinical situations. The patients were given: ATG (Immossar, Choay) 700 IU/kg/day for the first 15 days, then twice a week for another month, tapering steroids and azathioprine. T cell subsets were determined by Orthomune monoclonal antibodies. T suppressor function was studied by the Con-A enhancement test and the blastogeneic response to mitogens was measured. The normal trends of immunologically quiescent patients were evaluated at first (TAB 1).

TAB 1. Linear regressions of uncomplicated patients within 12 weeks.

T3%	y =	40	+	1.9x	r =	0.36	$P < 0.001$
T4%	y =	24	+	1.6x	r =	0.39	$P < 0.001$
T8%	y =	24			—		P NS
T4/T8	y =	1	+	0.09x	r =	0.44	$P < 0.001$
T supp. activ.	y =	59	−	1.3x	r =	−0.4	$P < 0.005$
Mitogen resp.	y =	15100	+	7700x	r =	0.51	$P < 0.001$

Acute rejection episodes were treated with steroid pulses and ATG During 15 rejections the following changes were observed (TAB 2).

TAB 2.	Quiescence prerej.	Crisis onset	Rej. treatment
T4/T8	1.54 ± 0.83	2.75 ± 1.23[b]	0.94 ± 0.56[c]
T4/T3%	53.4 ± 13.8	66.1 ± 15.4[a]	42.4 ± 23[c]
T8/T3%	38.3 ± 18.2	23.6 ± 9.8[b]	54.7 ± 22.1[c]
T supp. activ.	57.9 ± 25.4	20.1 ± 18[b]	21.9 ± 20.1

[a] $P < 0.02$ [b] $P < 0.01$ [c] $P < 0.001$

The frequency distribution of the T4/T8 ratio between rejection and quiescence was as follows (TAB 3).

TAB 3.	Rejection	Quiescence
T4/T8 < 1.800	2 (13.4%)	134 (76.6%)
T4/T8 ≥ 1.800	13 (86.6%)	41 (23.4%)
	$\chi^2 = 27.158$ $P < 0.0005$	

The T suppressor activity remained low about 4 weeks after a rejection episode. During bacterial infections, the T4/T8 ratio was usually high, especially after the first 4 weeks ($P < 0.01$). During documented viral infections, the T4/T8 ratio was low and often less than 1. HBs Ag positive patients, otherwise uncomplicated, displayed lower T4/T8 ratios than negative ones ($P < 0.0025$). No particular clinical and laboratory changes were observed in patients receiving prophylactic cimetidine. These findings were discussed.

Defective T-suppressor activity in type I membranoproliferative glomerulonephritis (MPGN-I). *G. Busnach, S. Bertoli, A. Fornasieri, R. Sinico, G. Fiorini, C. Guastoni, G. Barbiano di Belgiojoso, M. G. Lavagni, and B. Brando. Divisioni di Nefrologia, Ospedali Niguarda-Ca' Granda e S. Carlo Borromeo, and Banca del Sangue, Ospedale S. Carlo Borromeo, Milano, Italy.* T-cell subsets have been so far reported to be normal in MPGN. To investigate T-suppressor cell (Ts) activity, 12 patients with histologic diagnosis of MPGN-I have been studied: 6 males and 6 females; mean age, 37 ± 16.5 years. All patients had normal renal function; proteinuria did not exceed 2 g/24 h, and none had received immunosuppressive treatment during the year before. Duration of illness was 4 to 132 months. In 7 out of 12 serum C3 and/or C4 was low; CH50 was reduced in 6 out of 12 and an alternative pathway involved in 4 out of 12. Circulating immune complexes (S.P. conglutinin assay) were present in 2 out of 12. IgG were high in 5 out of 12 and low in 3 out of 12. T-cell subsets were analyzed by monoclonal antibodies of the OKT series. Ts function (TsF) was studied by the Con-A enhancement test (Con-A E) and with a specially designed assay where the response to pokeweed mitogen (PWM) is measured before and after physical OKT8+ cell removal by complement-mediated cell toxicity assay. In Table 1, MPGN-I patients are considered as a group, and in Table 2 normo (N) and hypocomplementemic (H) patients are compared.

Table 1

				Suppression %	
	R4/8	R4/3%	R8/3%	Con-A E	PWM-8 + Dep.
CONTROLS	1.8 ± 0.72	60.6 ± 11.21	31.1 ± 10.86	47.5 ± 15	40.6 ± 13.11
MPGN-I	1.2 ± 0.50	53.8 ± 17.41	44.2 ± 19.36	35.6 ± 22.5	18.3 ± 11.41
	$P < 0.025$	*P:* NS	$P < 0.05$	*P:* NS	$P < 0.005$

Table 2

N-MPGN	1.6 ± 0.44	68.4 ± 15.89	38.6 ± 14.9	31.3 ± 20.3	18.4 ± 14.12
H-MPGN	1.0 ± 0.34	43.5 ± 9.22	48.2 ± 22.22	35.9 ± 25.7	16.5 ± 10.09
	$P < 0.01$	$P < 0.005$	*P:* NS	*P:* NS	*P:* NS

The low OKT4/OKT8 ratio is due to a relative increase of OKT8+ cells ($P < 0.05$) and also to a reduction of OKT4+ cells in H patients ($P < 0.005$). TsF as studied with a specific OKT8+ cell-dependent test enabled us to detect a highly significant defect in MPGN-I, whereas Con-A E test, which is not strictly T-cell dependent, was unchanged. There is a discrepancy between T-cell subsets markers and T-cell functional activity: An increase of OKT8+ may obviate to their functional failure. Hypocomplementemia could be a marker of a defective TsF in MPGN-I, which is not affected by the duration of illness in patients with normal renal function and without nephrotic syndrome.

Epithelial polyanionic coat and changes of foot processes in courses of nephrotic syndrome in humans. *S. Casanova, U. Donini, P. Versura, and P. Zucchelli. Laboratorio Microscopia Elettronica e Servizio Anatomia Patologica, Divisione di Nefrologia e Dialisi, Ospedale M. Malpighi, Bologna, Italy.* Recent experimental studies on nephrotic syndrome presented the following data. The alterations of the glomerular epithelium seem to be correlated with the reduction of surface epithelial anionic charges. We studied, by light and electron microscopy, the affinity of the glomerular epithelium for colloidal iron on renal biopsy specimens of 12 patients with nephrotic syndrome. Six patients were suffering from membranous glomerulonephritis and the others had minimal change glomerulonephritis. In each patient we compared the degree of colloidal iron reaction with that of foot process fusion and with proteinuria. The colloidal iron reaction was carried out at pH 1.9. For electron microscopy 20-μ thick cryostat sections were briefly fixed then treated with colloidal iron thereafter postfixed with osmium and embedded in epoxy resins. For light microscopy cryostat sections of 4 to 6 μ were fixed in aceton for 5 min and then incubated in the colloidal iron solution. The presence of colloidal iron was evidenced through Prussian blue reaction. The control sections were prepared from an undamaged kidney portion obtained by a nephrectomy. By light microscopy, the reaction appeared to weaken in patients in which proteinuria was higher than 40 to 60 mg/mq/hr. By electron microscopy the reaction appeared unmodified even in the presence of a complete fusion of the foot processes and massive proteinuria. The weakened reaction for colloidal iron observed by light microscopy resulted from the reduction of the epithelial surface area caused by the foot process fusion. These data suggest that the colloidal iron reaction is not sufficiently sensitive to reveal small alterations of the surface epithelial charges.

Renal hypercalciuria (RH) and primary hypoparathyroidism (PH): Evidence for a common defect in distal tubular calcium reabsorption (TRCa). *G. Colussi, F. Malberti, M. Surian, A. Antonacci, A. Marni, G. Rombolà, G. Pontoriero, and L. Minetti. Divisione di Nefrologia, Ospedale Niguarda-Ca' Granda, Milano, Italy.* In previous studies we have shown that patients with RH (that is, a "diet-independent" or fasting type of idiopathic hypercalciuria) have normal tubular reabsorption (TRMg) of ultrafiltrable magnesium (UFMg) during fasting, while fractional excretion of ultrafiltrable Ca (UFCa) is higher than normal, indicating a reduced TRCa. The clearance ratio (ClUFCa/ClUFMg), which was lower than 1 in normal people (N) and in a group of diet-dependent hypercalciuric patients, was higher than 1 in all RH patients, as has been observed by micropuncture studies in early distal tubular fluid. Since Mg is poorly reabsorbed along the distal nephron (while TRCa and TRMg are tightly bound in the proximal tubule and Henle's loop), we suggested that hypercalciuria in RH is a consequence of reduced TRCa along the distal nephron. To verify such a hypothesis, we compared renal handling of UFCa and UFMg in 11 RH patients and in 8 patients with PH (idiopathic in four and postsurgical in four) made normocalcemic with either vitamin D (DHT in three patients, $25(OH)D_3$ in one patient) or calcium administration (i.v. and/or p.o.) in four patients.

PCa	UFCa		PMg	UFMg	CaE	FECa	MgE	FEMg	ClUFCa
	mg/dl				μg/dl GFR	%	μg/dl GFR	%	ClUFMg
N	9.52	4.95	2.00	1.24	68	1.59	34	2.80	0.55
	±0.74	±0.39	±0.21	±0.11	± 3	±0.88	± 9	±0.89	±0.14
RH	9.52	4.97	1.92	1.34	190[a]	4.17[a]	38	2.66	1.52[a]
	±0.51	±0.33	±0.12	±0.10	±100	±1.99	± 9	±0.67	±0.35
PH	9.50	4.76	1.85	1.26	220[a]	4.84[a]	33	2.72	2.18[a]
	±0.70	±0.50	±0.12	±0.09	±140	±3.25	±19	±1.72	±1.61

[a] $P < 0.001$ vs. N

Plasma values and filtered load per unit GFR of Ca and Mg, Mg excretion (MgE) and fractional excretion of UFMg (FEMg) did not differ in N, RH, and PH; Ca excretion (CaE), fractional excretion of UFCa (FECa), and ClUFCa/ClUFMg ratio were similarly increased both in RH and PH (range of the clearance ratio in N = 0.25 − 0.86, RH = 1.03 − 2, PH = 0.96 − 3.5); cAMP excretion was high in RH (4.4 ±

1.6 nmoles/dl GFR, $P < 0.001$ vs. N: 2.46 ± 0.72) and low in PH (1.49 ± 0.47, $P < 0.001$ vs. N). Renal phosphate threshold was high in PH (4.6 ± 0.39 mg/dl GFR, $P < 0.01$ vs. N: 3.83 ± 0.59) and low in RH (2.8 ± 0.59, $P < 0.001$ vs. N). In conclusion, *(1)* patients with PH (in whom PTH-dependent distal TRCa is reduced) kept normocalcemic and normomagnesiemic, show fasting hypercalciuria with normal TRMg and increased ClUFCa/ClUFMg ratio; *(2)* the pattern of renal handling of UFCa and UFMg is similar in RH and in PH and is consistent with a reduced TRCa along the distal nephron in RH; *(3)* low distal TRCa in RH is not due to a reduced parathyroid activity and could indicate either a primary tubular defect or the effects of a mild phosphate depletion state on the renal function.

Mechanism of impaired urinary concentration ability in chronic renal failure (CRF). *G. Conte, A. Dal Canton, G. Fuiano, M. Sabbatini, M. Terribile, and V. E. Andreucci. Cattedra di Nefrologia, II da Facoltà di Medicina, Università, Napoli, Italy.* In this study, the mechanism(s) by which urinary concentrating ability is impaired in CRF was investigated in three groups of patients with GFR of 20 to 50 ml/min. Group 1: Seven normotensive patients were studied in maximal antidiuresis. The clearance of creatinine (C_{cr}), sodium, urea, and osmolar clearance (C_{osm}) were measured while patients were receiving a normal dietary content of sodium and protein (A) and after a 1-week administration of low-sodium, low-protein diet (B). Group 2: The same clearance studies as in group 1 were performed in 12 normotensive and 12 hypertensive patients, both in antidiuresis and maximal water diuresis. In the latter condition, distal sodium delivery (Na_{dist}) could be calculated. Group 3: Eight hypertensive patients were studied in antidiuresis before and after drug-induced normalization of blood pressure. In group 1, in (B) C_{cr} was unchanged, fractional sodium excretion fell from 1.9 to 0.5%, and the filtered load of urea was reduced by 40%. No significant modification occurred in maximal urine osmolality (U_{osm}), nor in T^cH_2O. In group 2, in antidiuresis, U_{osm}, T^cH_2O and T^cH_2O/C_{osm} were significantly lower in hypertensive patients ($P < 0.01$; $P < 0.0125$, and $P < 0.05$, respectively). In water diuresis, instead, C_{H_2O}/dl GFR was similar in normotensives as hypertensives, despite that in the latter Na_{dist} was increased ($P < 0.05$). In group 3, normalization of blood pressure was not associated with any change in U_{osm}, nor in T^cH_2O. The results of this study indicate that neither ECF volume expansion, or increased nephron urea load play a critical role in reducing concentrating ability. This defect is better accounted for by intrinsic tubular damage that is enhanced in hypertensive patients.

IgA subclasses in circulating immune complexes, immunoglobulins and renal deposits of Berger and Henoch-Schönlein glomerulonephritis. *R. Coppo, B. Basolo, G. Mazzucco, M. R. Bulzomi, M. Messina, G. Camussi, G. Barbiano, R. Castello, and G. Piccoli. Cattedra di Nefrologia e Istituto Anatomia Patologica, Università, Torino; Divisioni di Nefrologia e Dialisi, Ospedali S. Giovanni, Torino e Niguarda-Ca' Granda, Milano, Italy.* The analysis of the IgA subclasses in plasma and renal tissue of patients affected by Berger and Henoch-Schönlein glomerulonephritis (GN), allows some insights into the pathogenesis, still controversial, of these nephropathies. IgA1- and IgA2-producing cells are almost equally represented in normal mucosas, whereas normal serum IgA contains 90% of IgA1 and 10% of IgA2. Conflicting data have been reported on kidney tissue. No parallel analyses of IgA subclasses in IgA containing circulating immune complexes (IgAIC) or in A immunoglobulins (IgA) have so far been presented. The authors studied 31 Berger GN and 15 Henoch-Schönlein GN by investigating: *(1)* IgAIC, IgA1IC, IgA2IC (original conglutinin solid phase assay); *(2)* IgA, IgA1, IgA2 serum levels (immunoenzymatic assay); *(3)* polymeric serum IgA (reduction-alkilation test); *(4)* IgA1 and IgA2 renal deposits (indirect immunofluorescence) on 15 kidney biopsy specimens. In both groups of patients mean serum IgAIC levels significantly greater ($P < 0.01$) than controls were observed, significantly higher in Henoch-Schönlein than in Berger GN (197 ± 328 vs. 61 ± 121 μg IgA aggr/ml; normal value (NV) 6 ± 9 μg IgA aggr/ml). The correlation of these data with clinical phases of activity was good. IgAIC significantly correlated with the amount of microscopic hematuria ($r = 0.26$, $P < 0.01$). Mean levels of IgA1IC and IgA2IC significantly greater than controls were observed in both groups (Berger GN: IgA1IC 145 ± 230 μg IgA aggr/ml, IgA2IC 158 ± 184 μg IgA aggr/ml; Henoch-Schönlein GN: IgA1IC 274 ± 444 μg IgA aggr/ml, IgA2IC 168 ± 213 μg IgA aggr/ml; nv: IgA1IC 18 ± 22 μg IgA aggr/ml, IgA2IC: 17 ± 33 μg IgA aggr/ml). In the active phase both IgA1 and IgA2IC significantly increased in Berger GN, whereas only IgA1IC increased in Henoch-Schönlein GN. The IgA1/IgA2 ratio in immunoglobulins was found to be similar with controls in either group of patients; it was not correlated with the IgA1IC/IgA2IC ratio. An increase on polymeric IgA was observed in 30% of Berger GN and in 50% of Henoch-Schönlein GN. Both IgA1 and IgA2 were represented in renal deposits: IgA2 deposits were heavy in 66% of the patients. These data suggest that IgA contained in IgAIC and renal deposits of Berger and Henoch-Schönlein GN are derived from mucosal plasma cells, in agreement with experimental models.

Presence of J chain in mesangial immune deposits (MID) of IgA nephropathy (IgANP). *U. Donini, S. Casanova, G. Maletta, and P. Zucchelli. Divisione di Nefrologia e Dialisi e Laboratorio Microscopia Elettronica, Servizio Anatomia Patologica, Ospedale M. Malpighi, Bologna, Italy.* The presence of J chain in MID of IgANP was investigated to identify polymeric IgA through immunohistochemical techniques. We studied renal biopsy specimens of 23 patients with Berger nephropathy (BNP), two with Henoch-Schönlein glomerulonephritis (HSGN) and two with IgA glomerulonephritis associated with alcoholic cirrhosis (IgAGNAC). As controls we used 10 patients with different nephropathies. Two with IgG deposits, six with IgM deposits, and two without deposits. Before the immunohistochemical reaction, all the kidney cryostat sections were treated for 20 min at 4°C with urea 6 M in glycine buffer 0.1 M, pH 3.2. Both goat and rabbit anti-J antisera (Nordic, The Netherlands) were used. By immunofluorescence these antisera were found to cross-react with IgG deposits in two control patients. To eliminate this cross-reaction we absorbed the anti-J antisera with purified human IgG. Ten patients, which we found to be J-positive by indirect immunofluorescence, were also studied by a direct immunofluorescence and peroxidase-antiperoxidase (PAP) method. Comparing the results of these various techniques, we decided to use PAP in our investigation. Our results indicate that J chain is present in MID of 17 out of 23 patients of BNP, in both the patients of IgAGNAC, in 1 out of 2 patients of HSGN and in 5 out of 6 control patients with IgM deposits. One control patient with a moderate degree of IgM deposits was J negative as were the remaining patients studied. The patients with BNP were separated into two groups: J-positive and J-negative. In comparing the clinical features of these groups, no significant differences were found. However the duration of the disease at the moment of biopsy was relatively shorter in the J-negative group. In fact the mean was 30.8 months for the J-positive and only 12.7 in the J-negative patients. Our results suggest that: *(1)* polymeric IgA are present in MID of the majority of BNP (73.9%); *(2)* nevertheless, the BNP could be caused even by monomeric IgA alone. This is most often verified in the starting phases of the illness. One can make the hypothesis that BNP, at the beginning of its natural history, is caused by monomeric IgA and only at a later stage does polymeric IgA intervene.

Role of monocytes in extracapillary glomerulonephritis (GN): Histochemical study in 15 patients. *F. Ferrario, G. Colasanti, A. Fornasieri, R. Sinico, E. Schiaffino, S. Nava, and G. D'Amico. Divisione di Nefrologia e Servizio di Anatomia Patologica, Ospedale S. Carlo Borromeo, Milano, Italy.* In studies concerning human and experimental GN, it has been shown recently that macrophages are involved actively both in extracapillary and intracapillary proliferative changes. To evaluate their presence in renal tissue and their possible role in disease, we used the "nonspecific esterase (NSE)" method in kidney biopsy sections from 15 patients affected by rapidly progressive GN (RPGN). All the patients presented circumferential crescents in 70% of glomeruli (cellular in 12, fibrotic in 3). Nine patients presented primary RPGN (six idiopathic, one postinfectious, one type II membranoproliferative, and one anti-GBM GN) and six suffered from systemic diseases (three SLE, three necrotizing angiitis). NSE-positive cells were measured separately in the crescents and the glomerular tuft, and expressed in the latter as the monocyte-index/glomerulus (MI/G). A number of NSE-positive cells was observed in all cellular crescents, whereas they were completely absent in fibrotic ones. The multinuclear giant cells of the crescents were strongly NSE-positive, confirming their similarity (or identity) with monocyte-macrophage cells. The

average MI/G was 5.6: It was significantly higher in patients with cellular crescents than in patients which showed mainly fibrotic crescents (6.6 vs. 1.9); as a whole, the MI/G was also higher in glomeruli with "active" crescents than in glomeruli without crescents (7.7 vs. 2.2). On the other hand, the average MI/G was similar in primary and systemic RPGN (6.7 and 6.4, respectively) and in patients with a different immunofluorescence pattern (negative 6.8; linear 7.6; granular 6.4). Repeat biopsies were performed in two patients who presented a favorable course, after 4 and 7 months, respectively: In these patients NSE-positive cells were no longer evident either in crescents or the glomerular tuft. In conclusion, our results strongly support experimental data on the "effector" role of monocyte-macrophages cells in the morphogenesis of extracapillary and intracapillary proliferative lesions in different forms of RPGN.

Complement (C) activation and factor VIII abnormalities in hemolytic-uremic syndrome (HUS) in childhood. *F. Ginevri, G. P. Tonini, A. Carrea, M. Pecorara, P. G. Mori, F. Perfumo, and R. Gusmano. Servizî di Nefrologia e Dialisi e di Ematologia-Oncologia, Istituto G. Gaslini, Genova, Italy.* In HUS C activation and more recently, Factor VIII complex (FVIIIC) abnormalities have been reported. Several hypothesis have been formulated for the understanding of C activation. The possibility of a proteolytic activity being responsible for C activation has been examined in this study. In nine children, aged 7 to 12 months to 5 years, during an acute phase of HUS, we have determined C3, C4, C1q, C1s, C5, C3act, and C3d by radial immunodiffusion, circulating immune complexes (CIC) by immunoenzymatic method; C3 splitting activity; FVIIIC by one stage clotting method; FVIIIRAG by Laurell electroimmuno assay; electrophoretic mobility of FVIIIRAG; and plasma sepharose CL6B gel chromatography. The results are: C3d increased in 8 out of 9 patients, C3 decreased in 4 out of 9, C4 decreased in 7 out of 9, C1q decreased in 2 out of 9, and C3act decreased in 4 out of 9; C1s and C5 were in the normal range. There was no evidence of CIC and C3 splitting activity. FVIIIRAG increased in 8 out of 9 and the ratio FVIIIRAG/FVIIIC was greater than 1.5 in 6 out of 9. Abnormal, symmetrical, more anodal FVIIIRAG electrophoretic mobility was found in 7 out of 9 patients. In the same patients a disappearance or reduction of high molecular weight forms ($>4 \times 10^6$D) has been observed by plasma gel filtration studies. Our results show that in HUS the activation of C occurs mainly via the classical pathway. In seven patients FVIIIRAG fragmentation and C activation suggest the possibility of proteolytic activity acting on both systems.

T-lymphocyte subpopulations defined by monoclonal antibodies in uremic patients on dialysis treatment. *M. Mandreoli, M. Briganti, G. Emiliani, A. Fabbri, L. Guerrini, E. Magni, A. Montanari, G. Monti, F. Lauria, and M. Fusaroli. Divisione di Nefrologia e Dialisi e Servizio di Anatomia Patologica, Ospedale S. Maria Croci, Ravenna; Istituto di Ematologia "Seragnoli" Università, Bologna, Italy.* Uremic patients are thought to present a variety of immunologic abnormalities affecting principally delayed hypersensitivity, homograft rejection, and T-cell response to mitogens, while B-cell functions remain relatively unaffected. In this study, using monoclonal antibodies (MoAbs) of OKT series (ORTHO), we evaluated the distribution of T-lymphocyte subsets in 23 patients on regular hemodialysis treatment (RDT) and in 10 patients on intermittent peritoneal dialysis (PD). We also investigated the mitogenic response to PHA. The aim of the work was to verify whether or not an intrinsic disorder of lymphocyte subpopulations exists in uremic patients.

	E^+ %	OKT_3^+ %	OKT_4^+ %	OKT_8^+ %	OKT_4^+/OKT_8	PHA *cpm* $\times 10^3$
HD	66	62	42	22	2	45261 $P < 0.001$
PD	71	65	43	26	1.7	42300 $P < 0.001$
Controls	72	70	43	25	1.7	72588

The proportion and absolute number of T-lymphocytes (E^+ and OKT_3^+ cells) resulted in normal in both groups. The analysis of T-cell subsets showed that both T-cells with helper/inducer (OKT_4^+ cells) and suppressor/cytotoxic (OKT_8^+ cells) phenotype were in the range, with OKT_4/OKT_8 ratio normally balanced. In contrast the in vitro proliferative response to PHA was reduced significantly in both patient groups as compared to healthy controls ($P < 0.001$). No correlation was found between the immunologic impairment and age, primitive renal disease, duration and schedule of treatment. Our data suggest that in uremic patients the impaired immune response may be due principally to functional disorder.

Arteriolar pathology in idiopathic glomerulonephritis. *G. Manganella and D. Turner. Insituto di Antomia Patologica, Università, Bari, Italy; Guy's Hospital, London, United Kingdom.* In some renal biopsy specimens (RB) from patients with idiopathic glomerulonephritis (IG) a certain amount of vascular damage may be observed as well as the glomerular and tubulo-interstitial lesions. To establish if some types of IG are more frequently associated than others with renal arteriolar damage 304 RB from patients with IG were examined. Araldite (0.5 μ) embedded sections stained with toluidine blue were observed by light microscopy and classified as positive or negative for arteriolar lesions. The biopsy specimens were subsequently subdivided according to the type of the IG, the age of the patient, above or under 40 years, and the presence or absence of hypertension as defined by a persistent diastolic of 95 mm Hg or more prior to the biopsy. This particular grouping was used to separate the vascular lesions resulting from aging or hypertension. The examination of the results reveals that 82.3% of renal biopsy specimens from normotensive patients under 40 with focal glomerulosclerosis (FGS) were positive for hyaline lesions, which compares with 19.3% positive RB of normotensive patients in the same age and pressure group affected by other forms of IG and, significantly, with 7.8% of positive normotensive young patients affected by minimal change glomerulonephritis (MCGN). One might think that the explanation for the greater frequency of hyaline subendothelial lesions in association with FGS lies in the high levels of serum lipids in the nephrotic syndrome (NS) associated with this form of IG; however, our results show that the lesions in question are uncommon in the MCGN also characterized by a NS with hyperlipidemia. The greater incidence of subendothelial hyaline deposits in FGS in the absence of hypertension and aging could explain the higher frequency and importance of the tubulo-interstitial lesions and the poor prognosis of this type of IG as compared with the more benign MCGN.

Effects of acetazolamide on calcium (Ca) and phosphate metabolism in nephrotic syndrome (NS). *P. Messa, G. Mioni, M. Adorati, D. Montanaro, and M. Messa. Divisione di Nefrologia e Dialisi, Ospedale Civile, Udine, Italy.* To better clarify the pathogenesis of reduced Ca absorption and hypocalciuria in NS, we studied 21 nephrotic patients (9 women and 12 men, aged 14 to 69 years) who had proteinuria in excess of 3 g/day (3.4 to 24.5) and normal renal function (C_{Cr} 80 to 147 ml/min). TmPi/GFR (measured by infusion methodology), fractional intestinal absorption of calcium (by double curve analysis), s-V-DBG, s-PTH-COOH, electrolytes, and acid-base balance in blood and urine were evaluated. On the basis of TmPi/GFR values, we divided our patients in two groups: group H ($N = 13$) with high (>4.4 mg/dl) TmPi/GFR (M ± SEM 5.13 ± 0.22) and group N ($N = 8$) with normal (between 2.2 and 4.4 mg/dl) TmPi/GFR (3.57 ± 0.09). Fractional intestinal absorption of calcium was significantly lower in group H when compared with group N (20.4 ± 1.9 vs. 47.6 ± 6.1%, $P < 0.001$; n.v. 30 to 55%); the values of fractional intestinal absorption of calcium were significantly correlated with TmPi/GFR values ($r = +0.63$, $P = <0.01$). Urinary Ca excretion also appeared lower in group H (15.6 ± 3.6 vs. 46.1 ± 15.2 mg/day $P < 0.05$). No significative difference was found regarding s-VDBG, s-PTH, C_{Cr}, u-Prot, and u-Na. Eleven out of these patients (five of group H and six of group N) received acetazolamide (ACZ), 250 to 375 mg/day for 12 days; after treatment all the above-mentioned parameters were re-evaluated. TmPi/GFR significantly fell in all treated patients (base 4.3 ± 0.3, ACZ 3.4 ± 0.1 mg/dl, $P < 0.01$), although the reduction was more

evident in group H (3.7 ± 0.12 vs. 5.1 ± 0.26, $P < 0.01$) than in group N (3.1 ± 0.11 vs. 3.6 ± 0.12, $P < 0.05$). Fractional intestinal absorption of calcium significantly increased in group H (41.4 ± 3.9 vs. 23.7 ± 3.3%, $P < 0.01$), whereas no significative change was observed in group N (52.9 ± 6.3 vs. 49 ± 7.4%, P = NS). The variations of intestinal absorption of calcium resulted well correlated with the variations of TmPi/GFR ($r = +0.72$, $P < 0.05$). ACZ also resulted in a striking calciuric effect (116.2 ± 20 vs. 32.8 ± 11 mg/day, $P < 0.01$), without any difference between the two groups; the calciuric effect appeared after 8 days of treatment and seemed poorly related to the sodiuretic and acidemic effects of ACZ, which appeared after 2 days of treatment, and seemed closely related to the increase in net acid excretion. These results suggest that the TmPi/GFR may play in some way a major role in affecting the levels of intestinal Ca absorption and urinary Ca excretion in nephrotic syndrome. Acetazolamide, through a reduction in the TmPi/GFR, may partly correct these metabolic derangements.

Does an increased splanchnic production of glucose contribute to glucose intolerance in CRI? *C. Robaudo, G. Deferrari, G. Garibotto, A. Canepa, G. Salvidio, G. Gurreri, and A. Tizianello. Cattedra di Nefrologia Medica e Servizio di Nefrologia, Istituto Scientifico di Medicina Interna, Università, Genova, Italy.* It is well established that a decreased peripheral glucose (G) utilization plays a major role in G intolerance frequently observed in chronic renal insufficiency (CRI); however, it is still controversial if an increased splanchnic production of G shares some responsibility in G intolerance. To investigate the latter possibility, G and aminoacid (AA) exchange across the hepatosplanchnic bed (HS) in the postabsorptive state was evaluated in six nondiabetic patients with CRI (GFR 16.3 ± 3.4 ml/min) and in six patients with normal renal function as controls. Both groups of patients had normal liver function tests and ate strictly comparable diets. Blood samples were obtained simultaneously from a peripheral artery and from a hepatic vein. Blood G was measured enzymatically and AA by an automatic analyzer. Hepatic blood flow was estimated by the continuous infusion technique employing Indocyanine green dye. In patients with CRI arterial G levels were not different from controls (4.51 ± 0.23 vs. 4.97 ± 0.17 mmoles/liter); HS G production and its ratio to insulin levels were not significantly changed in comparison with controls (521 ± 92 vs. 693 ± 167 μmoles/min and 38 ± 6 vs. 63 ± 14, respectively). The sum of the uptakes of the individual gluconeogenic AA was not different between the two groups (186 ± 19 μmoles/min in controls and 245 ± 43 in CRI). The ratio of the AA utilized, expressed as G equivalents, to G produced by the HS, showed a tendency to increase in CRI; thus, in this condition, both G production by the HS and gluconeogenesis from AA are normal. Furthermore, the normal ratio of G production to insulin suggests an unmodified hepatic sensitivity to insulin. These data indicate that G intolerance in CRI is not the consequence of an altered G metabolism across the HS and confirm indirectly the view that G intolerance depends on a peripheral resistence to insulin.

Hematoliquoral changes induced by dialysis. *C. Ronco, S. Biasioli, S. Chiaramonte, A. Fabris, M. Feriani, G. D'Andrea, P. Parisen, E. Pisani, and G. La Greca. Servizi di Nefrologia e di Neurologia, Ospedale S. Bortolo, Vicenza, Italy.* This study has been designed to investigate the cerebrospinal fluid (CSF) changes induced by dialysis (HD). Two lumbar punctures (before and after HD) were performed on 10 consenting patients (eight male, two female, mean age 38.5 years) maintained by regular intermittent HD (4 hr × 3/week; 1 sm^2 filter) from almost 1 year. CSF and venous blood samples were taken simultaneously for SMAC24 and acid-base status determination. CSF pre- and postdialytic values were, respectively: Na = 154 – 146, K = 3.4 – 2.9, Cl = 120 – 116 mEq/liter; BUN = 72.3 – 63.7, Creatinine = 4 – 3.5, glucose = 57 – 56, Ca = 4.3 – 4.2, P = 1.7 – 1.6 mg%, osmolality = 334 – 328 mOsm/kgH_2O; pH = 7.35 – 7.35, P_{CO_2} = 42.2 – 39.9, HCO_3^- = 22.1 – 21.4 mEq/liter. Pre- and postdialytic CSF/plasma ratios were, respectively: Na = 1.10 – 1.05, K = 0.63 – 0.80, Cl = 1.16 – 1.15, BUN = 0.80 – 1.52; creatinine = 0.33 – 0.58, Ca = 0.5 – 0.4, P = 0.33 – 0.46, osmolality = 0.98 – 1.0, pH = 0.99 – 0.98, P_{CO_2} = 1.19 – 1.22, HCO_3^- = 1.12 – 0.92. Student's t test for paired data was applied showing a significant variation in CSF composition and in CSF/plasma ratios induced by dialysis. A parallel decrease in brain density (cerebral CT evaluskop) of about 25% from baseline values was registered. In the meantime, also the trend of plasma and CSF aminoacid (AA) levels was investigated, before and after HD. So, it could be demonstrated that AA plasma levels change remarkably during the treatment, while CSF concentrations change scarcely. So CSF/plasma ratios are influenced by HD: These changes do not correlate with CSF/plasma ratios regarding the above-mentioned solutes. Neutral, basic, and acid AA seem to show different changes of CSF/plasma ratios during dialysis: This could be a new approach to evaluate the dialysis encephalopathy syndrome.

Calcium and phosphate renal handling and parathyroid activity in pregnancy. *M. Surian, G. Colussi, F. Malberti, A. Marni, P. Cosci, G. Pontoriero, G. Rombola, T. Fogazzi, A. Aroldi, G. Graziani, and L. Minetti. Servizio di Emodialisi, Ospedale Maggiore, Lodi; Servizî di Nefrologia, Ospedali Niguarda-Ca' Granda e Policlinico, Milano, Italy.* During pregnancy there is an increase of calcium (Ca) and phosphate (P) intestinal absorption due to early elevated $1,25(OH)_2D_3$ serum levels. Data on parathyroid activity (PTH) are conflicting: PTH levels have been found high, normal, or low. Moreover, the role of the kidney in the change of Ca and P metabolism has not well clarified. We have performed a longitudinal study in 15 healthy pregnant women on a free diet and without Ca supplements or any kind of drugs in three different periods of pregnancy (1:6th to 16th; 2:17th–28th; 3:29th week–term) and 3 months after delivery or the end of lactation (C). Serum ionized calcium (Ca^{++}) (1:4.54 ± 0.32 mg/dl; 2:4.4 ± 0.21; 3:4.4 ± 0.24; C:4.54 ± 0.22), fractional excretion of Ca^{++} (FECa: 1:1.2 ± 0.7%; 2:1 ± 0.6; 3:1.2 ± 0.7, C:1.3 ± 0.7), plasma P, renal threshold of P (1:3.5 ± 0.6 mg/dl GFR, 2:3.8 ± 0.6, 3:3.9 ± 0.7, C:3.6 ± 0.6), PTH (COOH-terminal fragment) (1:1.16 ± 0.48 ng/ml, 2:1.3 ± 0.6, 3:1.13 ± 0.69, C:1 ± 0.38) and urinary excretion of cAMP (1:2.2 ± 0.8 nmoles/dl GFR, 2:2.7 ± 1.3, 3:2.7 ± 0.8, C:2.5 ± 1) did not change significantly during pregnancy. As a consequence of augmented GFR, a significant increase of Ca^{++} filtered load (1:7.1 ± 1.9 mg/min, 2:8.2 ± 3.3, 3:7.2 ± 2.7, C:5.8 ± 1.5, $P < 0.05$ and <0.02 in 1 and 2 versus C) and of urinary Ca excretion (UCa/UCr, 1:0.2 ± 0.08, 2:0.2 ± 0.09, 3:0.16 ± 0.07, C:0.13 ± 0.05, $P < 0.01$ and <0.02 in 1 and 2 versus C) was observed. In five women neither plasma cAMP levels (1.64 ± 0.52 nmoles dl) nor nephrogenous cAMP production (1.38 ± 0.7 nmoles/dl GFR) were significantly different from C (1.86 ± 0.28 and 1.12 ± 0.74, respectively) during gestation. Our data on the basis of normal values of PTH, urinary cAMP excretion, renal threshold of P and tubular reabsorption of Ca, evaluated as fractional excretion of Ca^{++}, argue against a "physiologic" hyperparathyroidism in pregnancy, as suggested by other authors. The augmented GFR is responsible for an increase of Ca-filtered load and, as a consequence, for an elevated urinary calcium excretion, which unmodified is the tubular fractional reabsorption of calcium.

Acid-base disorders in idiopathic hypercalciuria. *N. Tessitore, E. Valvo, G. Panzetta, A. Lupo, C. Loschiavo, A. Fabris, L. Oldrizzi, L. Gammaro, C. Rugiu, N. Iurino, V. Ortalda, and G. Maschio. Cattedra e Divisione di Nefrologia Medica, Università e Istituti Ospitalieri, Verona, Italy.* In view of the increasing evidence of multiple renal tubular defects in patients with calcium nephrolithiasis, especially in those with hypercalciuria, we have evaluated the renal regulation of acid-base balance in 16 adult patients (11 males, 5 females) with "renal leak" hypercalciuria through the oral short NH_4Cl loading-test (0.1 g/kg body wt) and an intravenous bicarbonate loading-test ($NaHCO_3$ 0.5 M to 5 mEq/min). All patients had normal GFR, concentrating capacity, plasma potassium, phosphate, and i-PTH values and no evidence of infection or obstruction of the urinary tract. We made diagnosis of incomplete proximal renal tubular acidosis (RTA) in six patients (37.5%) according to high HCO_3 fractional excretion at normal plasma bicarbonate levels (>5%) and reduced Tm HCO_3^-/dl GFR (<2.1 mEq/dl GFR). Three patients (18.7%) had incomplete distal RTA, according

to high urinary pH values (≥5.4) and low ammoniuria (<25 μEq/min) under NH_4Cl loading and to the failure to increase the urine-blood P_{CO_2} gradient during bicarbonate infusion (<26 mm Hg). In two of them the proximal and distal defects were associated. When compared to the patients with normal acid-base status, the ones with RTA showed comparable Na^+, K^+, Cl^-, and phosphate renal handling but significantly increased basal urinary pH (6.6 ± 0.2 vs. 5.6 ± 0.1) and stone activity (1.1 vs. 0.5 stones/patient/year): These findings seem to suggest a pathophysiological role of the acidifying defect in calcium-containing stone formation, possibly through higher urinary pH values. Under bicarbonate loading we found a linear relationship between Na^+ and Ca^{++} fractional excretions in controls as well as in hypercalciurics: In the two groups the slope was the same (0.40 to 0.45), but the intercept was different (0.21 and 1.31). This finding suggests a normal renal tubular handling of calcium in hypercalciuria, even if the urinary calcium excretion is always set to a higher value. Moreover, no difference between patients with or without RTA was observed. In conclusion, our study shows a high incidence of incomplete acidifying defects, mainly proximal, in "renal leak" hypercalciuria and suggests that these defects are unrelated to the renal calcium handling.